



Office de la propriété
intellectuelle
du Canada

Un organisme
d'Industrie Canada

Canadian
Intellectual Property
Office

An Agency of
Industry Canada

PT / CA 00/00801

28 JUL 2000 28.07.00
REC'D 09 AUG 2000

Bureau canadien
des brevets
Certification

La présente atteste que les documents
ci-joints, dont la liste figure ci-dessous,
sont des copies authentiques des docu-
ments déposés au Bureau des brevets.

Canadian Patent
Office
4
Certification

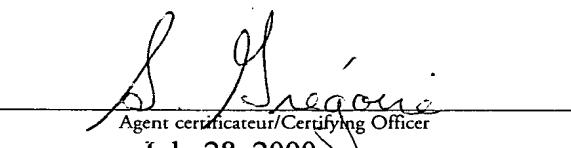
This is to certify that the documents
attached hereto and identified below are
true copies of the documents on file in
the Patent Office.

CA 00/801

Specification and Drawings, as originally filed, with Application for Patent Serial No:
2,274,873, on July 6, 1999, by **LUC VARIN AND SATINDER GIDDA**, for "Methods
and composition for Modulating Flowering"

PRIORITY
DOCUMENT

SUBMITTED OR TRANSMITTED IN
COMPLIANCE WITH RULE 17.1(a) OR (b)


Agent certificateur/Certifying Officer

July 28, 2000

Date

Canada

(CIPO 68)

OPIC  CIPO

METHODS AND COMPOSITIONS FOR MODULATING FLOWERING**FIELD OF THE INVENTION**

5

The invention relates to methods to control flowering time in plant species by increasing or lowering the levels of active 12-OH-jasmonate and/or its glucoside in plants.

10 DESCRIPTION OF THE PRIOR ART

Flower formation is an inductive process by which the growing tip of the plant switches from a vegetative to a reproductive mode of development. In some plant species, environmental signals such as, photoperiod, light quality and quantity, vernalization, and nutrient and water availability trigger this change in the developmental fate of the shoot apical meristem. In other species, the timing of flowering is mainly under the control of endogenous signals that are believed to appear when the plant reaches a certain size or a certain stage of development (For a review, see Levy and Dean, 1998).

20

The results of several studies suggest that the flower inductive process occurs in response to a signal molecule that is translocated from the leaves to the shoot apical meristem where it induces floral transition. For example, the results of several grafting experiments demonstrate that a flowering signal could be transmitted through the graft union from a flowering induced shoot or even from induced leaves to a non-induced graft partner. The chemical signal has been referred to in the literature as "florigen". Several research years were lost hunting for florigen in induced plant extracts, and its chemical structure is still unknown today (For a review, see Aukerman and Amasino, 1998).

25

30 The results of the present studies on the *Arabidopsis thaliana* gene AtST2, suggest that in fact, the florigen is 12-OH-jasmonic acid, also known as tuberonic

acid (Figure 1). 12-OH-jasmonic acid was first isolated from the leaves of *Solanum tuberosum* (potato) during a search for of a tuber-inducing compound (Yoshikara et al. 1989). Later, it was shown that the level of the tuber-inducing substance (12-OH-jasmonate) was increasing at the onset of tuber formation. The biosynthesis of 5 12-OH-jasmonate has not been studied, but we can predict that an hydroxylase converts jasmonic acid or methyl jasmonate to the 12-hydroxylated compound.

Tazaki (Japanese kokai 2-92220 (A) published April 3 1990, patent application no 63-242432, filed September 29, 1988), Yoshihara et al. (1989), 10 Matsuki et al. (1992), and Koda and Okazawa (1988) all disclosed that treating potato stem fragments with jasmonates in culture induces tuber formation. In addition, Ryan and Farmer (International patent application WO 91/18512, published December 12, 1991) disclosed treating plants with jasmonates to induce production of defense proteins. However, none of these documents disclose or 15 suggest the application of 12-OH-jasmonate to induce flower formation, nor was there a link made between flower formation and 12-OH-jasmonate.

SUMMARY OF THE INVENTION

20 A need exists for an effective method to control flowering time in crop plants and in plants with horticultural value without decreasing yield or modifying plant morphology.

25 It is an object of the present invention to provide compositions and methods for modulating flowering time in plants.

More particularly, it is an object of the invention to provide a composition to induce flowering in plants. The composition for inducing flowering according to the invention comprises at least one compound selected from the group consisting of:
30 - inhibitors of a gene named *AtST2* or inhibitors of ortholog(s) of this gene in plant species other than *Arabidopsis thaliana*;
- inhibitors of an 12-OH jasmonate sulfotransferase;

- activators of an 12-OH jasmonate hydroxylase;
- compounds selected from the group consisting of 12-OH jasmonate, glucoside of 12-OH jasmonate, 12-OH methyl-jasmonate, glucoside of 12-OH methyl-jasmonate, 11-OH jasmonate, glucoside of 11-OH jasmonate, 11-OH methyl-jasmonate, glucoside of 11-OH methyl-jasmonate, jasmonic acid, Me-jasmonate and mixtures thereof. Preferably, the inhibitor of the *AtST2* gene is selected from the group consisting of antibodies specific to the *AtST2* gene product and/or antisense mRNA to the transcribed *AtST2* gene; the inhibitor of the 12-OH jasmonate sulfotransferase enzyme is selected from the group consisting of antibodies specific thereto or of chemical compounds inhibiting the enzyme activity.

10 It is also an object of the present invention to provide a method for inducing flowering in plants which comprises the step of applying an effective amount of one of the above-mentioned early flowering compositions to a plant.

15 It is a further object of the invention to provide early flowering transgenic plants, methods to produce the same and uses thereof, the early flowering transgenic plants being selected from the group consisting of:

- transgenic plants which express lower levels of the *AtST2* gene or its ortholog(s) than a non-transgenic plant;
- transgenic plants which comprise a DNA sequence encoding the *AtST2* gene or its ortholog(s) in the antisense orientation;
- transgenic plants which demonstrate a lower level 12-OH jasmonate sulfotransferase activity than a non-transgenic plant;
- transgenic plants which demonstrate a higher level of 12-OH jasmonate hydroxylase activity than a non-transgenic plant;
- transgenic plants which demonstrate a higher level of at least one compound selected from the group consisting of 12-OH jasmonate, glucoside of 12-OH jasmonate, 12-OH methyl-jasmonate, glucoside of 12-OH methyl-jasmonate, 11-OH jasmonate, the glucoside of 11-OH jasmonate, 11-OH methyl-

jasmonate, glucoside of 11-OH methyl-jasmonate jasmonic acid, methyl jasmonate and mixtures thereof.

According to this aspect of the present invention there is provided a method
5 for producing an early flowering transgenic plant with reduced endogenous or
existing level of 12-OH jasmonate sulfotransferase activity, said method
comprising stable transforming of a cell of a suitable plant with a nucleic acid
molecule which comprises a sequence of nucleotides encoding or complementary
10 to a sequence encoding the *AtST2* gene, regenerating a transgenic plant from the
cell and where necessary growing said transgenic plant under conditions sufficient
to permit the expression of the nucleic acid. According to another preferred
embodiment, the method to induce early flowering time comprises the step of
generating a loss of function of the *AtST2* gene (or its ortholog(s) in other plant
species), preferably by mutating in the *AtST2* gene. Alternatively, *AtST2* gene
15 function inhibition is achieved by the expression in the plant of an antibody specific
for the 12-OH jasmonate sulfotransferase.

It is an additional object of the invention to provide a composition to retard
flowering in plants. The composition for retarding flowering according to the
20 invention comprises at least one compound selected from the group consisting of:

- activators of a gene named *AtST2* or activators of ortholog(s) of this gene in
plant species other than *Arabidopsis thaliana*;
- activators of the 12-OH jasmonate sulfotransferase;
- inhibitors of the 12-OH jasmonate hydroxylase;
- 25 - antibodies specific to the 12-OH jasmonate hydroxylase.

Furthermore, it is an object of the invention to provide late flowering
transgenic plants, methods to produce the same and uses thereof, the late
flowering transgenic plant being selected from the group consisting of:
30

- transgenic plants which express higher levels of the *AtST2* gene or its
ortholog(s) than a non-transgenic plant;

- transgenic plants which demonstrate a higher level of 12-OH jasmonate sulfotransferase activity than a non-transgenic plant;
- transgenic plants which comprise a DNA sequence encoding the jasmonate 12-hydroxylase gene in the antisense orientation;

5 - transgenic plants which demonstrate a lower level of 12-OH jasmonate hydroxylase activity as compared to a non-transgenic plant; and

- transgenic plants which demonstrate a lower level of at least one compound selected from the group consisting of 12-OH jasmonate, glucoside of 12-OH jasmonate, 12-OH methyl-jasmonate, glucoside of 12-OH methyl-jasmonate, 11-OH jasmonate, the glucoside of 11-OH jasmonate, 11-OH methyl-jasmonate, glucoside of 11-OH methyl-jasmonate, jasmonic acid, and Me-jasmonate.

10

In a preferred embodiment, the method to retard flowering time in plants
15 comprises the step of inserting the coding sequence of *AtST2* (or its ortholog(s) from other plant species) in the sense orientation under the control of a constitutive or inducible promoter.

Another aspect of the present invention contemplates a method for
20 producing a late flowering transgenic plant with reduced endogenous or existing level of 12-OH jasmonate hydroxylase activity, said method comprising stably transforming a cell of a suitable plant with a nucleic acid molecule which comprises a sequence of nucleotides encoding or complementary to a sequence encoding the 12-OH jasmonate hydroxylase activity, regenerating a transgenic plant from the cell and where necessary growing said transgenic plant under conditions sufficient to permit the expression of the nucleic acid.

Other preferred embodiments to the method to retard flowering comprise
the step of treating plants by inhibiting the synthesis of 12-OH-jasmonate.
30 Preferably this inhibition is achieved by the application of an inhibitor of the 12-OH-hydroxylase enzymatic activity, or by the expression in transgenic plants of the jasmonate 12-hydroxylase gene in the antisense orientation, or by generating a

loss of function of the jasmonate 12-hydroxylase gene, preferably by mutation. Alternatively, the inhibition is achieved by the expression in the plant of an antibody specific for the 12-OH-hydroxylase.

5 In another preferred embodiment, the method to retard flowering, comprises the steps of stable transforming of a cell of a suitable plant with a nucleic acid molecule which comprises the coding sequence of *AtST2* (or its ortholog(s) in other plant species) in the sense orientation under the control of a constitutive or inducible promoter, regenerating a transgenic plant from the cell and where
10 necessary growing said transgenic plant under conditions sufficient to permit the expression of the nucleic acid.

Other objects and features of the invention will become apparent upon reading the following, non-restrictive description of several preferred embodiments
15 thereof, made with reference to the enclosed examples.

DETAILED DESCRIPTION OF THE INVENTION

The Applicants have now discovered a method to induce flowering in plants
20 by the exogenous application of 12-OH-jasmonate and/or its glucoside. Furthermore, they characterized the biological function of a gene from *A. thaliana* (*AtST2*, see figure 2) which encodes an enzyme that inactivates the biological activity of 12-OH-jasmonate by sulfonation. The expression of this gene in transgenic *A. thaliana* results in plants with delayed flowering time. Furthermore,
25 the length of the delay was found to correlate with the level of expression of *AtST2*.

The following definitions are provided in order to provide clarity as to the intent or scope of their usage in the specification and claims.

30 The term a plant as used herein refers to a whole plant or a part of a plant comprising, for example, a cell of a plant, a tissue of a plant, an explant, or seeds

of a plant. This term further contemplates a plant in the form of a suspension culture or a tissue culture including, but not limited to, a culture of calli, protoplasts, embryos, organs, organelles, etc.

5 The term **transgenic plant** or **transgenic plant tissue** as used herein refers to a plant or plant tissue stably transformed with a foreign gene introduced into the genome of the individual plant cells using genetic engineering. The term **transformed plant** or **transformed plant tissues** as used herein refers to introduction of a foreign DNA into a plant or plant tissue and expression of the
10 DNA in the plant or plant tissue.

The term **genetic engineering** as used herein refers to the introduction of foreign, often chimeric, genes into one or more plant cells which can be regenerated into whole, sexually competent, viable plants which can be self-pollinated or cross-pollinated with other plants of the same species so that the
15 foreign gene, carried in the germ line, can be inserted into or bred into agriculturally useful plant varieties.

20 The term **antisense** as used herein refers to nucleic acids molecules capable of regulating expression of the corresponding gene in a plant. An antisense molecule as used herein may also encompass a gene construct comprising the structural genomic or cDNA gene or part thereof in reverse orientation relative to its or another promoter.

25 The term **ortholog** as used herein refers to a molecule having at least 50%, more preferably at least 55%, even more preferably at least 60%, still more preferably at least 65-70%, and yet even more preferably greater than 85% similarity at the level of nucleotide or amino acid sequence to at least one or more regions of the nucleotide or amino acid sequence set forth in Figure 2 and wherein
30 the nucleic acid encodes or is complementary to a sequence which encodes an enzyme having AtST2 activity. It should be noted, however, that nucleotide or amino acid sequences may have similarities below the above given percentages

and yet still encode an AtST2-like molecule and such molecules may still be considered within the scope of the present invention where they have regions of sequence conservation.

5 The term **flowering** as used herein refers to the appearance of the flower. The term **flowering** in conjunction with the term **time** refers to the appearance of the first petal.

10 Accordingly, the terms **activate**, **induce** or **increase** in conjunction with the term **flowering**, refer to the reduction of the time of vegetative growth before the appearance of the first petal. To the opposite, the terms **retard** or **delay** in conjunction with the term **flowering**, refer to the increase of the time of vegetative growth before the appearance of the first petal.

15 The term **effective amount** as used herein refers to the amount or concentration of a suitable compound that is administered to a plant such that the compound induces early flowering or delays flowering in a plant.

20 As stated herein before, the present invention contemplates many objectives. In the practice, two alternate approaches are preferably used to achieve the objects of the invention:

1) **A chemical approach:**

25 A) Early flowering is induced by the application of a composition comprising 12-OH-jasmonate and/or its glucoside (see example 1).

30 These two compounds can be applied in a pure form or as a mixture of jasmonates including 11-OH-jasmonate and its glucoside, jasmonic acid and Me-jasmonate to plants. The inducing composition may further comprise an inert carrier or a solvent such as water, oils or alcohol, and also comprise active agents such as growth regulators. The inducing composition may also be formulated with

emulsifying agents in presence or absence of fungicides or insecticides, if required. The precise amount of compound employed in the practice of the present invention will depend upon the type of response desired, the formulation used and the type of plant treated.

5

Furthermore, the inducing composition may further comprise an inhibitor of the AtST2 protein to prevent the *in-vivo* inactivation of the flower-inducing molecule by sulfonation, a reaction catalyzed by the AtST2 protein (or its ortholog(s) in other plant species).

10

B) Early flowering is induced by the application of a composition comprising 12-OH-jasmonate and/or its glucoside to transgenic plants expressing the AtST2 gene (or its ortholog(s) in other plant species) in the antisense orientation to prevent the inactivation of the flower inducer by sulfonation.

15

C) Flowering time is delayed by the application of a composition comprising a chemical inhibitor of the jasmonate 12-hydroxylase. This retarding composition may further comprise an inert carrier or a solvent such as water, oils or alcohol, and also comprise active agents such as growth regulators. The retarding composition may also be formulated with emulsifying agent in presence or absence of fungicides or insecticides, if required. The precise amount of compound employed in the practice of the present invention will depend upon the type of response desired, the formulation used and the type of plant treated.

25 2) A biotechnological approach:

A) Flowering time is delayed by the production of transgenic plants expressing AtST2 (or ortholog(s) from other species) under the control of a constitutive or inducible promoter. Alternatively, the pattern of expression of the AtST2 gene in *A. thaliana* or its ortholog(s) in other plant species can be modified by a gain of function mutation.

The *AtST2* gene from *Arabidopsis thaliana* encodes a sulfotransferase that sulfonates 12-OH-jasmonic acid and 11-OH-jasmonate with high specificity. *AtST2* exhibits high affinity for its substrate with a *K_m* value of 11 μM for 12-OH-jasmonate and 60 μM for 11-OH-jasmonate. The nucleotide sequence of *AtST2* is 5 available in the *Arabidopsis thaliana* database at Stanford University (clone number M0J9 and 119G6T7) and in the GeneBank database under the accession number AB010697 (nucleotide 55016 to 53747). The nucleotide and deduced amino acid sequences of *AtST2* are illustrated in Figure 2.

10 As shown in Example 2, the expression of this gene in transgenic *Arabidopsis thaliana* affects flowering time. Expression of *AtST2* in the sense orientation under the control of the CAMV35S promoter results in transgenic plants which exhibit delayed flowering time as compared with the non-transformed control plants. Furthermore, the length of the delay was found to correlate with the 15 level of expression of the transgene. In spite of the delay in flowering time, the growth behavior and the size of the transgenic plants could not be distinguished from the non-transformed control plants.

20 B) Flowering time is delayed by the introduction in transgenic plants of the gene encoding the jasmonate 12-hydroxylase in the antisense orientation.

C) Early flowering is induced by the production of transgenic plants expressing *AtST2* (or its ortholog(s) in other plant species) in the antisense orientation as 25 shown in Example 3. Alternatively, the *AtST2* gene (or its ortholog(s) in other plant species) can be inactivated by a loss of function mutation. When *AtST2* is expressed in the antisense orientation under the control of the CAMV35S promoter, transgenic *A. thaliana* plants exhibit early flowering time when treated with Me-jasmonate as compared with non-transformed control plants. Apart from early flowering, the growth behavior and the size of the transgenic plants could not 30 be distinguished from the non-transformed control plants.

The details of the construction of transgenic plants are known to those skilled in the art of plant genetic engineering and do not differ in kind from those practices which have previously been demonstrated to be effective in tobacco, petunia and other model plant species.

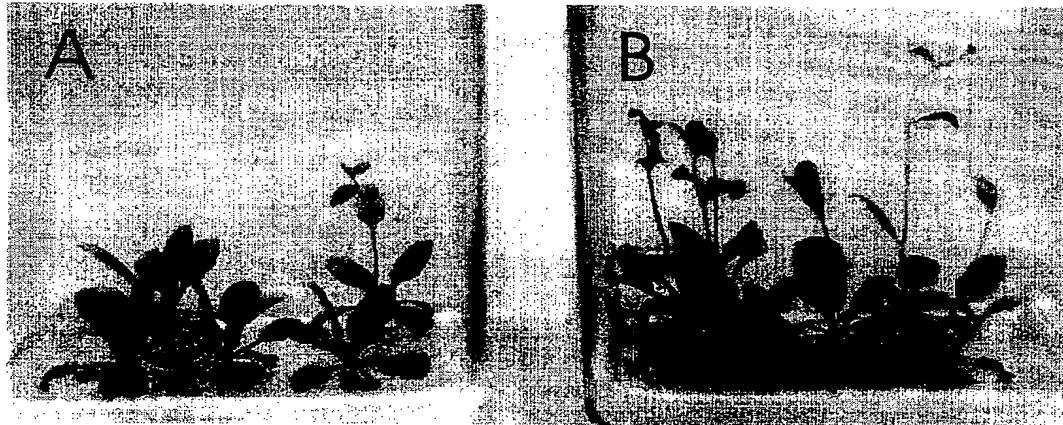
5

EXAMPLES

The following examples are illustrative of the wide range of applicability of the present invention. The invention is not restricted to the control of flowering in 10 *Arabidopsis thaliana* but can be applied to various plant species. It should readily occur that the recognition of activation or retardation using the compositions, and methods according to the present invention in connection with other plants not specifically illustrated herein is readily within the capabilities of one skilled in the art. The following examples are intended only to illustrate the invention and are not 15 intended to limit its scope. Modifications and variations can be made therein without departing from the spirit and scope of the invention.

Example 1: *A. thaliana* plants were treated with 10 μM 12-OH-jasmonate to demonstrate its effectiveness as a flower inducer.

20



A) Control 18 days old *A. thaliana* (Col0) plants

B) 18 days old *A. thaliana* (Col0) plants treated with 12-OH-jasmonate

18 Days old *A. thaliana* (Col 0) plants were treated with 10 µM 12-OH-jasmonate in (A) or with water in (B) for a period of 6 days. The results show that treated plants flowered earlier than non-treated plants. The plants were grown in magenta boxes containing phytoagar and vitamins in a growth chamber under a
5 16-hour photoperiod, at a day temperature of 24 °C and a night temperature of 20°C.

These results are of a great economic importance since they prove that it is possible to induce flower formation by the exogenous application of 12-OH-jasmonate and/or its glucoside to crop plants. It will allow to induce early flowering when required by a simple application of a flower inducer.
10

Example 2: *A. thaliana* was transformed with the *AtST2* gene under the control of the CaMV35S promoter in the sense orientation to demonstrate the effectiveness
15 of expressing this gene to delay flowering time.

Description of the vector:

The EcoR1-HindIII cassette of the pBI-525 vector (Datta, R.S. et al) was inserted at the same sites in the pBI-101 vector (Clontech). The resulting vector
20 called pBI-101-525 contained two CaMV 35S minimal promoters in tandem followed by an AMV translational enhancer, a NOS terminator and a kanamycin resistance gene. *AtST2* cDNA was cloned both in the sense and the antisense orientation at the BamHI site in the polylinker lying downstream of the AMV enhancer.
25

Agrobacterium transformation:

A. tumefaciens strain GV3101 pMP90 was transformed with the *AtST2*-pBI-101-525 sense and antisense constructs by the method of Gynheung et al.

30 Arabidopsis transformation:

A. thaliana plants of ecotype Columbia (ColO) were transformed with Agrobacterium containing the *AtST2* gene in the sense orientation by the vacuum

infiltration method as described previously by Bencholt et al. *A. thaliana* ecotype C24 were transformed with the pBI-101-525 vector containing the *AtST2* gene in the antisense orientation by the root explant method described by Valvekans et al. The seeds collected from the T_0 plants were surface sterilized and transformants 5 selected on MS salt medium containing vitamins and supplemented with 50 microgram per ml of kanamycin.



WT S1 S3 S5 S6 S7 S9 S10

10 WT (Col 0) = non-transformed line after 27 days of growth.
S1 to S10 = Transgenic lines expressing constitutively *AtST2* after 27 days of growth.

15 All the transgenic lines exhibit delayed flowering as compared with non-transformed plants. The plants were grown in soil in a growth chamber under a 16-hour photoperiod, at a day temperature of 24 °C and a night temperature of 20 °C.

Example 3: *A. thaliana* was transformed with the *AtST2* gene under the control of the CAMV35S promoter in the antisense orientation to demonstrate the 20 effectiveness of expressing this gene to induce early flowering (see Example 2 for experimental details).

Transgenic line 7-2-5

Non-transformed control plant



7-2-5

W

The plants were treated with Methyl-jasmonate for 9 days. The plants were
5 grown in magenta boxes containing phytoagar and vitamins in a growth chamber
under a 16-hour photoperiod, at a day temperature of 24 °C and a night
temperature of 20 °C.

10

REFERENCES

Throughout this application, references are made to articles of scientific literature which are listed below:

15 Aukerman, M.J. and Amasino, R.M. (1998) Floral induction and florigen. *Cell*, **93**:
491-494.

20 Benchtold, N., Ellis, J., Pelletier, G. CR Acad Sci paris, Life Sciences. In planta *Agrobacterium* mediated gene transfer by infiltration of adult *Arabidopsis thaliana* plants. **316**;1194-1199 (1993).

Dalta, R., Bekkaoui, F., Hammerlindl, J. K., Pilate, G., Dunstan, D., Crosby, W. Improved high level expression in plants using an AMV RNA4 untranslated leader sequence. *Plant science*. **94**: 139-149 (1993).

Gynheung, An., Paul, R., Amita M., and Sam B. Binary Vectors, Plant molecular Biology Manual.A3:1-19 (1988).

5 Koda, Y. et al. (1988) Detection of potato tuber-inducing activity in potato leaves and old tubers. *Plant Cell Physiol.*, **29**: 969-974.

Levy, Y.Y., and Dean, C. (1998) The transition to flowering. *The Plant Cell* **10**, 1973-1989.

10

Matsuki, T. et al. (1992) The influences of jasmonic acid methyl ester on microtubules in potato cells and formation of potato tubers. *Biosci. Biotech. Biochem.* **56**: 1329-1330.

15 Valvekans, D., Van Montagu, M.V., Van Lijsebettens, M.V. *Agrobacterium tumefaciens* mediated transfomation of *Arabidopsis thaliana* root explants by using kanamycin selection. *Proc Natl Acad Sci USA*. **85**:5536-5540 (1988).

Yoshihara, T. et al. (1989) Structure of a tuber-inducing stimulus from potato

20 leaves (*Solanum tuberosum* L.). *Agric. Biol. Chem.* **53**: 2835-2837.

CLAIMS:

1- A method to induce flowering by the application of 12-OH-jasmonate and/or its glucoside to plants.

5

2- A method to induce flowering by the application of 12-OH-jasmonate and/or its glucoside in presence of an inhibitor of the 12-OH-jasmonate sulfotransferase.

3- A method to induce flowering by the application of a mixture containing 12-OH-
10 jasmonate (and/or its glucoside) and 11-OH-jasmonate (and/or its glucoside).

4- A method to induce flowering by the application of a mixture containing 12-OH-
jasmonate (and/or its glucoside) and 11-OH-jasmonate (and/or its glucoside) in
presence of an inhibitor of the 12-OH-jasmonate sulfotransferase.

15

5- A method to induce flowering by the application of 12-OH-jasmonate and/or its glucoside to transgenic plants expressing the *AtST2* gene (or its functional homolog from species other than *Arabidopsis thaliana*) in antisense under the control of a constitutive promoter.

20

6- A method to induce flowering by the application of a mixture containing 12-OH-
jasmonate (and/or its glucoside) and 11-OH-jasmonate (and/or its glucoside) to
transgenic plants expressing the *AtST2* gene (or its functional homolog from species other than *Arabidopsis thaliana*) in antisense under the control of a
25 constitutive promoter.

7- A method to induce flowering by the application of 12-OH-jasmonate and/or its glucoside to transgenic plants expressing the *AtST2* gene (or its functional homolog from species other than *Arabidopsis thaliana*) in antisense under the
30 control of an inducible promoter.

8- A method to induce flowering by the application of a mixture containing 12-OH-jasmonate (and/or its glucoside) and 11-OH-jasmonate (and/or its glucoside) to transgenic plants expressing the *AtST2* gene (or its functional homolog from species other than *Arabidopsis thaliana*) in antisense under the control of an
5 inducible promoter.

9- A method to retard flowering time by the construction of transgenic plants expressing the *AtST2* gene (or its functional homolog from species other than *Arabidopsis thaliana*) under the control of a constitutive promoter.
10

10- A method to retard flowering time by the construction of transgenic plants expressing the *AtST2* gene (or its functional homolog from species other than *Arabidopsis thaliana*) under the control of an inducible promoter.

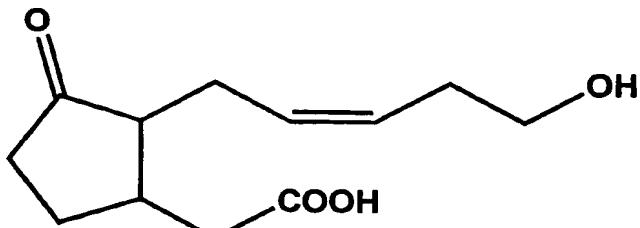
15 11- A method to retard flowering by the application of an inhibitor of the jasmonate 12-hydroxylase.

12- A method to retard flowering by the construction of transgenic plants expressing the gene encoding the 12-hydroxylase in the antisense under the
20 control of a constitutive promoter.

13- A method to retard flowering by the construction of transgenic plants expressing the gene encoding the 12-hydroxylase in the antisense under the control of an inducible promoter.

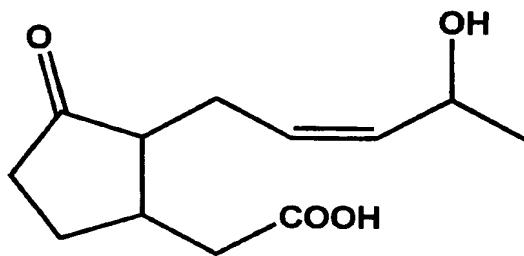
25

Figure 1: Chemical structures of 12-OH-jasmonate and 11-OH-jasmonate.



5

12-hydroxy-jasmonate



10

11-hydroxy-jasmonate

15

Figure 2. Nucleotide and deduced amino acid sequence of AtST2**Nucleotide sequence of AtST2**

5 atggctacctcaagcatgaagagcattccaatggcgatcccaagtttctccatgtgtcac
 aagctcgagtccttaagaaggcaaaactcgacgtcccggaaagccgaagaagatgaa
 gggctaagctgcgagttccaagagatgttggattcttcttaaggagagaggatggaga
 actcggttacccatccatccaaagggtttggccaaagccaaagagattcaagccatc
 atgtcttccaaaaacattccaatccctcgaaaacgcgtcgccccaccatacc
 10 aaatccggtacaacctggctaaaagcttaactttcaccatccttaaccgtcaccggtt
 gatccgggtgcctcgagtaccaaccaccctttcacttccaaaccctcatgaccc
 ccttcttcgagtacaagcttacgccaacggagatgttcccgatctctcgggtctagcc
 agtccaagaacgttcgcaacccacttaccgtcggtccctaaaggaaacgatcgagaaa
 cccggtgtgaaggctgtacttgcgcggaaacccttgcacacattcatcttcgtgg
 15 cattacaccaacaacatcaaatccgagtcagtgagccagtcttgctagaccaagcttt
 gatctgtattgccggggagtgatcggtttggccgtttggaaacacatgttgggatac
 tggagagagagcttgaagagaccagagaaagtcttctttaaggatcgaggatctcaaa
 gacgacatcgagaccaacttgaagaggcttgcaccccttagagcttcccttaccgaa
 gaagaggaacgaaaggagttgtgaaggctatcgccgagctgttagcttcgagaatctg
 20 aagaagttggaggtgaacaagtcaaaacaactcgatcaagaactttgagaatcgattctt
 ttccggaaaggagaagttagtgcattgggttaactattgtcaccttccacaagtggaaaga
 ttgtcagccttagtggatgacaagtttaggtggatctggctcacttccaggtttagctaa

Deduced amino acid sequence of AtST2

25 MATSSMKSIP MAIPSFSMCH KLELLKEGKT RDVPKAEEDE GLSCEFQEML
 DSLPKERGWR TRYLYLFQGF WCQAKEIQAI MSFQKHFQSL ENDVVFATIP
 KSGTTWLKAL TFTILNRHRF DPVASSTNHP LFTSNPHDLV PFFEYKLYAN
 GDVPDLSGLA SPRTFATHLP FGSLKETIEK PGVKVVYLCR NPFDTFISSW
 30 HYTNNIKSES VSPVLLDQAF DLYCRGVIGF GPFWEHMLGY WRESLRPEK
 VFFLRYEDLK DDIETNLKRL ATFLELPFTE EERKGVVKA IAE LCSFENL
 KKLEVNKSNK SIKNFENRFL FRKGEVSDWV NYLSPSQVER LSALVDDKLG
 GSGLTFRLS*